PRODUCTION OF CLEAVAGE BY SUPPRESSION OF THE POLAR BODIES IN ARTIFICIALLY ACTIVATED EGGS OF URECHIS

ALBERT TYLER

(From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, California, and the William G. Kerckhoff Marine Laboratory, Corona del Mar, California)

It was suggested in an earlier paper (Tyler, 1931*b*) that those eggs which do not ordinarily divide as a result of artificial activation could be made to do so by suppression of the polar bodies. This was based on the fact that upon activation by means of dilute sea water only those eggs divide that extrude no polar bodies. The other type of egg produced by artificial activation behaves very much like the normally fertilized egg in its initial reactions to the treatment, extrudes two polar bodies, but does not divide. The results of the experiments reported here show that the polar bodies can be suppressed in such eggs by means of a second treatment with dilute sea water and that the eggs then divide.

When *Urechis* eggs are treated with dilute sea water the percentage of the activated eggs that divide after various lengths of exposure bears an inverse relation to the total percentage of activation (Tyler, 1931*b*). Thus exposures resulting in 100 per cent activation give no cleavage, and the eggs are all of the type that extrudes both polar bodies. This simplifies the task of re-treating such eggs, since no unactivated eggs and no eggs of the type that divides are present in the dishes. The percentage of activation can be determined at about ten minutes after the initial treatment, and since the first polar body appears at thirty minutes at room temperature there is ample time for the second treatment.

The first attempts at polar body suppression were made by means of anesthetics such as ether, phenyl urethane, and chloretone in various concentrations. Low temperature was later tried, as was also hypertonic sea water. These agents gave variable results, and in general although the polar bodies were suppressed while the eggs remained under treatment they often appeared later when the eggs were removed to normal sea water.

Dilute sea water was then tried and this was found to be quite an effective agent for suppressing the polar bodies and producing cleavage.

The concentrations used were 50 and 55 per cent sea water. Higher concentrations generally failed to suppress the polar bodies and lower concentrations appeared to injure the eggs.

The Second Treatment with Dilute Sea Water

In these experiments the eggs were first treated for various lengths of time with 30 or 40 per cent sea water. The length of exposure resulting in 100 per cent activation is known fairly well from previous experiments, and so treatments ranging about the optimum time were used. The dishes were then examined to determine which actually

Table I

Re-Treatment with 55 per cent sea water. Eggs first treated for 2 minutes with 30 per cent sea water gave 100 per cent activation and all eggs later showed two polar bodies and no cleavage. First polar body out at 30 minutes; second at 40 minutes. *p.b.* = polar body.

Time after first treatment	Length of second treatment	Cleaved			Uncleaved		
		0 p. b.	1 p. b.	2 p. b.	0 p. b.	1 p. b.	2 p. b.
minutes	*minutes*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
25	5	0	0	0	0	0	100
25	10	2	3	0	0	2	89
25	15	20	6	0.2	2	3	69
25	20	36	2	0	34	12	16
25	25	71	2	0	18	9	0
25	30	94	1	0	3	1	1
25	35	96	2	0	1	1	0
25	40	88	0	0.1	7	3	2
40	5 to 40	0	0	0	0	0	100
55	5 to 40	0	0	0	0	0	100

showed 100 per cent activation. Large samples of eggs were then transferred from such dishes at various times after the beginning of the first treatment to 50 or 55 per cent sea water. The eggs were usually exposed to the second treatment for 5 to 40 minutes. They were examined in the dilute sea water and later in normal sea water to determine whether or not the polar bodies were suppressed by the treatment. The usual precautions in regard to the amount of water transferred with the eggs, etc. were taken.

The results of one such series of experiments are given in Table I. The eggs were first treated with 30 per cent sea water and an exposure of two minutes was found to give 100 per cent activation. All of the

eggs in this two-minute dish later showed two polar bodies. The first polar body appeared at 30 minutes and the second at 40 minutes after the first treatment. A sample of eggs was transferred to 55 per cent at 25 minutes after the first treatment; that is, 5 minutes before the first polar body was due to appear, and treated for various lengths of time. About an hour later the percentage of cleavage was determined and also the presence or absence of polar bodies. As shown in the table, when the eggs are exposed for 5 minutes to the second treatment with dilute sea water the polar bodies appear and no cleavage is obtained as in the controls. However, upon longer exposures fewer of the eggs show two polar bodies, and after exposure of 25 minutes or more practically none of the eggs show two polar bodies. At the same time the percentage of cleavage increases from zero to 98 per cent. The great majority of the cleaved eggs have no polar bodies. A small percentage of the divided eggs have one polar body (column 4 in the table) and a very few of the divided eggs show two polar bodies.

When the second treatment is applied at 40 or at 55 minutes after the first treatment (*i.e.*, after extrusion of the second polar body) the results are the same as for the control eggs—none of the eggs divide.

Fourteen series of experiments of the type illustrated by Table I were run and all gave similar results. Cleavage was obtained when the second treatment was applied before the time of extrusion of the first polar body and was continued until after the time of extrusion of the second polar body. When the eggs were given equivalent treatments at any time after the second polar body had appeared no cleavage was obtained. Cleavage was sometimes obtained when the treatment was applied after the extrusion of the first polar body. Eggs were also isolated after the second treatment according to the number of polar bodies they showed, and of 200 eggs examined cleavage was obtained in 90 per cent of the eggs that showed no polar bodies, 15 per cent of the eggs with one polar body, and none of the eggs with two polar bodies.

At the first cleavage the doubly treated eggs divided into two or three cells. Of 400 eggs on which counts were made 65 per cent divided into two cells and 35 per cent into three cells. Cleavage often stopped in the four-cell stage. Large numbers of abnormal top and bottom-swimmers but no normal embryos were obtained from the re-treated eggs.

It is evident then that when the polar bodies are suppressed by means of a second treatment the eggs are then capable of division. Suppression of one polar body appears to be less effective in this regard than suppression of both.

Cytological preparations were made of the doubly treated eggs according to the method previously described (Tyler, 1932) in order to determine the behaviour of the chromosomes and centrosomes. Eggs were removed for preservation directly from the dilute sea water and also after their return to normal sea water. In the eggs preserved within twenty minutes after the application of the second treatment the achromatic figure was generally not visible, and the chromosomes appeared as condensed bodies, similar to their metaphase condition. They formed a single group at the pole of the egg. In eggs removed at later times from the dilute sea water the chromosomes were often found in two groups of about 12 to 18 each although generally they appeared in one group of about 18 scattered about in the polar region. When the eggs were returned to normal sea water at 30 minutes after the second treatment and later preserved, they first showed two chromosome groups which were generally associated with two separate asters. At later stages the eggs showed a single group of chromosomes, presumably due to the fusion of the two separate groups. The asters are usually not visible at this time. At the time of cleavage an amphiaster develops, and the chromosomes are seen distributed irregularly about the spindle. The first cleavage divides the chromosomes irregularly and counts of anaphase groups ranged from 8 to 40, the two groups sometimes containing equal numbers and at other times radically different numbers of chromosomes. Later stages were not followed.

The examination of the cytological preparations shows that when the polar bodies are suppressed, the chromosomes first separate into two groups which later come together and distribute themselves more or less irregularly about the first cleavage spindle.

Discussion

An important question involved in the cleavage of artificially activated eggs concerns the origin of the amphiaster. The parthenogenesis experiments of Herlant (1918), Fry (1925), and others on the echinoderm egg are generally taken to mean that central bodies and asters may arise *de novo* and either combine or divide to form an amphiaster. Although this argues against Boveri's view of the genetic continuity of the central bodies, more evidence has recently been presented in its favor from other sources (Sturdivant, 1931; Wilson and Huettner, 1931; Pollister, 1930; and Johnson, 1931). The parthenogenesis experiments on *Cumingia* (Morris, 1917; Heilbrunn, 1925) and on *Urechis* (Tyler, 1931*a*) show that cleavage is obtained when the eggs fail to extrude polar bodies. The question arises as to whether in such cases

the first cleavage spindle develops *de novo* or whether it is directly continuous with the first polar spindle. Evidence of the similarity of the first cleavage spindle of such eggs and the normal first polar spindle has been previously presented (Tyler, 1932). In the results presented here it was shown that suppression of the polar divisions enables eggs to divide which would not ordinarily do so. The cytological work is insufficient to determine whether when the polar divisions are suppressed the first maturation spindle is converted into the first cleavage spindle. The two separate asters observed when the polar bodies are suppressed may have been derived from the poles of the first maturation spindle or may have arisen *de novo*. The fact that similar treatments applied after polar body extrusion do not produce such effects favors the former view, but in the absence of more detailed cytological evidence the question as to the origin of amphiaster in the doubly treated eggs still remains open.

The double treatment used here obviously differs from Loeb's classical double treatment for sea-urchin eggs. In these experiments the agent used for the second treatment was of the same type as that used for the first; and its effect was to enable eggs to divide by suppressing the polar bodies. Moreover, Just (1922) has clearly shown that in the sea-urchin egg only a single treatment is necessary, whereas for several different agents used for single treatments on *Urechis*, the optimally activated eggs do not divide. Thus hypertonic sea water alone gives similar results to hypotonic sea water. Hypertonic sea water was also tried on optimally activated eggs after the extrusion of the polar bodies, but no cleavage occurred.

Summary

1. The polar bodies can be suppressed in artificially activated eggs of *Urechis* by means of a second treatment with dilute sea water.

2. The treatment must be applied before the time of extrusion of the first polar body and continued until after the time of extrusion of the second.

3. The eggs in which the polar bodies are thus suppressed undergo cleavage whereas ordinarily they would not do so.

4. Similar second treatments applied after the time of the extrusion of the second polar body do not induce cleavage.

BIBLIOGRAPHY

Fry, H. J., 1925. Asters in Artificial Parthenogenesis. I. Origin of the amphiaster in eggs of Echinarachnius parma. *Jour. Exper. Zoöl.*, **43**: 11.

Heilbrunn, L. V., 1925. Studies in Artificial Parthenogenesis. IV. Heat parthenogenesis. *Jour. Exper. Zoöl.*, **41**: 243.

HERLANT, M., 1918. Comment agit la solution hypertonique dans la parthénogenèse expérimentale (Methode de Loeb). I. Origine et signification des asters accessoires. *Arch. de zool. expér. et gén.*, **57**: 511.

JOHNSON, H. H., 1931. Centrioles and Other Cytoplasmic Components of the Male Germ Cells of the Gryllidae. *Zeitschr. f. wiss. Zool.*, **140**: 115.

JUST, E. E., 1922. Initiation of Development in the Egg of Arbacia. I. Effect of hypertonic sea-water in producing membrane separation, cleavage, and top-swimming plutei. *Biol. Bull.*, **43**: 384.

LOEB, J., 1913. Artificial Parthenogenesis and Fertilization. University of Chicago Press.

MORRIS, M., 1917. A Cytological Study of Artificial Parthenogenesis in Cumingia. *Jour. Exper. Zoöl.*, **22**: 1.

POLLISTER, A. W., 1930. Cytoplasmic Phenomena in the Spermatogenesis of Gerris. *Jour. Morph.*, **49**: 455.

STURDIVANT, H. P., 1931. Central Bodies in the Sperm-Forming Divisions of Ascaris. *Science*, **73**: 417.

TYLER, A., 1931*a*. The Production of Normal Embryos by Artificial Parthenogenesis in the Echiuroid, Urechis. *Biol. Bull.*, **60**: 187.

TYLER, A., 1931*b*. The Relation between Cleavage and Total Activation in Artificially Activated Eggs of Urechis. *Biol. Bull.*, **61**: 45.

TYLER, A., 1932. Chromosomes of Artificially Activated Eggs of Urechis. *Biol. Bull.*, **63**: 212.

WILSON, E. B., and A. F. HUETTNER, 1931. The Central Bodies Again. *Science*, **73**: 447.